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(54) Title: COMPOSITION AND METHOD FOR TREATMENT OF AGING SKIN

(57) Abstract

The present invention relates to a topical composition for application to the skin comprising an effective amount of ADP, AMP or oxaloacetic acid, or a combination thereof, with a cosmetically or pharmaceutically acceptable carrier. The compositions of the invention can be used to increase the energy level of cells, particularly skin cells, and to treat and prevent the symptoms of aging in the skin.

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COMPOSITION AND METHOD FOR TREATMENT OF AGING SKIN

Field of the Invention

The invention relates to cosmetic and pharmaceutical compositions. In particular, the invention relates to cosmetic and pharmaceutical compositions which enhance skin cell energy and rejuvenate aging skin cells.

Background of the Invention

As anyone into their middle years recognizes, the skin undergoes a number of mostly undesirable changes as it ages. These nature of these changes has now been fairly well documented on gross, tissue, and cellular levels. It is all too readily apparent to the naked eye that the skin of older individuals is drier, has more lines and wrinkles, and is generally more slack or loose than it was in youth. On the tissue level, many of these gross changes can be attributed to alterations in the production of collagen, which is greatly reduced in quantity, as well as being wasted and degraded in appearance. Interestingly, age-related changes can also be detected at the cellular and molecular level. As fibroblasts age, their cellular functions begin to decline. In particular, it has been observed that aging human fibroblasts *in vitro* are less responsive to stimuli which induce migration of cells in culture, and also replicate less frequently. Muggleton-Harris and Aroian, Somat. Cell Genet. 8: 41-50, 1982. It has been suggested that these changes are somehow related to age-related deficiencies in the normal cycle of ATP utilization and restoration. However, studies on the loss of replication have been said to indicate that it is not related to deficiencies in the ATP-restoring pathways (Goldstein et al. J. Cell Phys. 112: 419-424, 1982). Muggleton-Harris and

DeFuria(In Vitro Cell. Devel. Biol. 21: 271-276, 1985) in treating cells with metabolic poisons, showed that there is an age-dependent change in cells' ATP content and turnover rate, leading to the suggestion that aging cells lose some of their capacity to utilize ATP. Whatever the reason, however, the end result is an apparently inevitable reduced production in collagen, resulting in the typical skin atrophy seen in aged skin, and this loss of cellular function and decline in cell appearance appear to inextricably intertwined.

It would be desirable to be able to alter this inexorable descent into cellular old age, in the hopes of prolonging the "youthful" energy exhibited by younger fibroblast cells. However, to date, there has been no remedy for this aspect of the aging of skin cells. The present invention now provides a means for enhancing the ATP levels of aged fibroblasts.

Summary of the Invention

The invention provides a method for increasing ATP levels in aging cells which comprises applying to the skin an effective amount of ADP, AMP, or oxaloacetic acid, or a combination thereof. The method is particularly useful in increasing energy in skin cells, particularly fibroblasts. The invention also provides cosmetic and pharmaceutical compositions for topical application to the skin comprising effective amounts of ADP, AMP, or oxaloacetate, or a combination thereof in a cosmetically or pharmaceutically acceptable carrier.. As used herein, the terms "ADP", "AMP" and "oxaloacetic acid" shall include safe and effective derivatives thereof which retain qualitatively the same activity.

Detailed Description of the Invention

The present invention is based on an observation that the extrinsic provision of ADP, AMP or oxaloacetic acid(oxaloacetate) to skin cells results in an increase of cell energy present in the cells so treated. In particular, when ADP, AMP or oxaloacetic acid is provided to normal fibroblasts in culture, ATP levels can be increased substantially. Thus, use of ADP and/or AMP and/or oxaloacetic acid as active agents in the treatment and prevention of skin aging is indicated.

The compositions of the invention comprise effective amounts of ADP, AMP or a combination thereof, in combination with a cosmetically or pharmaceutically acceptable carrier. By "effective amount" is meant that amount of the active agent which can increase the amount of ATP in a treated cell at least about 10%, preferably at least about 20%, more preferably at least about 30%, and most preferably at least about 40%, relative to untreated cells. In a typical composition, the concentration of the active agent will be from about 0.001-10%, preferably about 0.01-5%, by weight of the total composition; the concentration may be varied depending upon the intended frequency of use of the composition, lower concentrations being employed with more frequent applications.

For topical application, the active components) can be formulated with a variety of cosmetically and/or pharmaceutically acceptable carriers. The term "pharmaceutically or cosmetically acceptable carrier" refers to a vehicle, for either pharmaceutical or cosmetic use, which vehicle delivers the active components to the intended target and which will not cause harm to humans or other recipient organisms. As used herein, "pharmaceutical" or "cosmetic" will be understood to encompass both human and animal pharmaceuticals or cosmetics. Useful carriers

include, for example, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, or mineral oil. Methodology and components for formulation of cosmetic and pharmaceutical compositions are well known, and can be found, for example, in Remington's Pharmaceutical Sciences, Eighteenth Edition, A.R. Gennaro, Ed., Mack Publishing Co. Easton Pennsylvania, 1990. The carrier may be in any form appropriate to the mode of delivery, for example, solutions, colloidal dispersions, emulsions(oil-in-water or water-in-oil), suspensions, creams, lotions, gels, foams, mousses, sprays and the like.

The active agents of the invention can be used alone to increase the energy level of skin cells generally, so as to delay or reverse the onset of the cellular symptoms of aging. By "aging", as used in the present specification and claims, is meant both photoaging, i.e., the premature aging which occurs as the result of excessive exposure to UV rays, and chronological aging, i.e., the naturally occurring, normal aging of the skin which occurs over time, even without prolonged sun exposure. The compositions of the invention are particularly suited for enhancing the energy levels of fibroblasts and keratinocytes in both normal and aging skin. Increase in energy levels of fibroblasts can be expected to delay or reverse the decrease in collagen and elastin production that characterizes aged fibroblasts. Similarly, the increase in energy levels of keratinocytes can be expected to result in a delay in the thinning of the epidermis observed in aging skin. The use of the active agents of the invention is not limited to skin cells, however, but may also be expected to aid in energy increase in, for example, aging muscle or other connective tissue cells.

Treatment of aging skin, or prevention of aging in normal, non-aged skin is preferably achieved by regular application of the composition over a period of time. A preferred method of obtaining the benefits of the composition is via chronic topical application of a safe and effective amount of a composition containing the mixture, to prevent or delay development of skin damage which may result from photo- or chronoaging, or to prevent worsening of or to reverse already established damage. It is suggested as an example that topical application of the composition, in an amount of from about 0.1 $\mu g/cm^2$ to 2 mg/cm^2 of exposed skin, be performed from about once per week to about 4 or 5 times daily, preferably from about 3 times a week to about 3 times daily, most preferably about once or twice per day. By "chronic" application, it is meant herein that the period of topical application may be over the lifetime of the user, preferably for a period of at least about one month, more preferably from about three months to about twenty years, more preferably from about six months to about ten years, more preferably still from about one year to about five years, thereby resulting in the treatment or prevention of the external signs of photoaging and chronological aging. However, the compositions of the invention may also be used on a less frequent basis, for example, once or twice a month, as a sort of "spa treatment" with a higher level of active component provided on these occasions.

The external signs of aging that the composition may alleviate include, but are not limited to, fine and deep lines and wrinkles, skin atrophy, reduction in skin thickness, changes in skin pigmentation, and reduction in hair growth.

In their function as general anti-aging agents, the actives of the invention can also be combined with other

anti-aging or skin-enhancing agents. For this purpose, the actives of the invention can be combined, for example, with one or more of the following products: alpha- or beta-hydroxy acids, such as lactic acid, glycolic acid, citric acid, alpha-hydroxyoctanoic acid, alpha-hydroxydecanoic acid, alpha-hydroxylauric acid, tartaric acid, glucouronic acid, galactouronic acid, alpha-hydroxybutyric acid, alpha-hydroxyisobutyric acid, malic acid, mandelic acid, pyruvic acid, and tartronic acid, and salicylic acid; retinoids, such as retinol, retinyl acetate, retinyl palmitate, retinyl butyrate, retinyl oleate, retinyl linoleate, and retinoic acid; and DHEA and derivatives thereof.

The formulation, in addition to the carrier and active agents, also can comprise other components which may be chosen depending on the carrier and/or the intended use of the formulation. Additional components include, but are not limited to, water soluble colorants (such as FD&C Blue #1); oil soluble colorants (such as D&C Green #6); water soluble sunscreens (such as Eusolex 232); oil soluble sunscreens (such as octyl methoxycinnamate); particulate sunscreens (such as zinc oxide); antioxidants (such as BHT); chelating agents (such as disodium EDTA); emulsion stabilizers (such as carbomer); preservatives (such as methyl paraben); fragrances (such as pinene); flavoring agents (such as sorbitol); humectants (such as glycerine); waterproofing agents (such as PVP/Eicosene Copolymer); water soluble film-formers (such as hydroxypropyl methylcellulose); oil-soluble film formers (such as Hydrogenated C-9 Resin); cationic polymers (such as Polyquaternium 10); anionic polymers (such as xanthan gum); vitamins (such as tocopherol); and the like. As will be apparent, the compositions can be therapeutic products, ADP and/or AMP and/or oxaloacetic acid being the sole actives,

or in combination with other actives. However, the compositions can also be a makeup products, for example, a lipstick, foundation, concealer, bronzer, blush, eyeshadow and the like.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example I.

This example illustrates the increase in cell energy levels produced by treatment of cells with ADP or AMP.

Normal human dermal fibroblasts are grown to confluence in flasks, and then divided into three different treatment sets. Testing is done at the point at which the cells have undergone 32 population doublings. The treatments are with ADP(Sigma), AMP(Yamasa Shoyu Co., Inc.), and creatine monophosphate(Sigma), each at five different concentrations ranging from 0.01-1.0 mM. The growth medium serves as a control.

The cells are treated for a period of 2 hours, and then trypsinized, washed and resuspended to $6X10^6$ cells/ml. The cells are treated with a releasing agent, sodium lauryl sulfate, which causes the ATP contained within the cells to be released. The releasate is then added to a solution containing luciferin and luciferase. Luciferase utilizes the energy provided by free ATP to convert luciferin to oxyluciferin. The light released by this reaction is read on a spectrophotometer; the amount produced is proportional to the amount of ATP available. The ATP levels are calculated from an ATP standard curve, and then normalized on a per cell basis. The results are shown in Table 1.

TABLE 1

Sample		ATP(pg/cell)	% increase
Media control		14.7	
ADP	0.01mM	14.0	-5
	0.05mM	17.7	20
	0.10mM	18.7	27
	0.50mM	23.0	56
	1.00mM	20.7	41
AMP	0.01mM	14.7	0
	0.05mM	19.0	29
	0.10mM	19.0	29
	0.50mM	20.0	36
	1.00mM	19.3	31
Creatine	0.01mM	16.7	14
	0.05mM	13.3	-9
	0.10mM	14.7	0
	0.50mM	12.7	-14
	1.00mM	15.0	2

The results shown above indicate that ADP increases ATP levels in fibroblasts in a dose dependent manner. AMP is also found to increase the ATP levels of treated cells, but not to the same extent as ADP, and not in a dose dependent manner. Creatine monophosphate, however, does not significantly increase ATP levels at any dose tested. The maximal increase achieved by ADP is +56% at 0.5mM, whereas the maximal increase achieved by AMP is +36% at 0.5mM.

Example 2

This example illustrates the increase in cell energy levels produced by treatment of cells with oxaloacetic acid.

Normal human dermal fibroblasts are grown to subconfluence in flasks, treated with oxaloacetic acid for two hours with three samples, having concentrations of 0.05 mM, 0.50mM and 1.0 mM. The cells are then trypsinized, washed and resuspended to $6X10^6$ cells/ml. The treated cells have undergone four passages. The cells are further treated and analyzed as described in the previous example, with the amount of ATP calculated at the various dosages. The results are shown in Table 2.

TABLE 2

Sample		ATP(pg/cell)	% increase
Media control		1.67	
OxAc	0.05mM	1.92	15
	0.50mM	2.58	55
	1.0mM	2.17	30

These data show that oxaloacetic acid at all concentrations increased ATP levels in treated cells, with a maximal increase of 55% observed at the 0.1mM dose.

What we claim is:

1. A cosmetic or pharmaceutical composition for topical application to the skin comprising an effective amount of ADP, AMP or oxaloacetic acid, or a combination thereof, with a cosmetically or pharmaceutically acceptable carrier.

2. The composition of claim 1 comprising from about 0.001 to about 10% of ADP, AMP, oxaloacetic acid or a combination thereof.

3. The composition of claim 2 comprising from about 0.1 to about 5% of ADP, AMP, oxaloacetic acid or a combination thereof.

4. The composition of claim 1 comprising ADP.

5. The composition of claim 1 comprising AMP.

6. The composition of claim 1 comprising oxaloacetic acid.

7. A method for increasing the energy level in cells which comprises applying to the skin an effective amount of the composition of claim 1.

8. The method of claim 7 in which the cells are skin cells.

9. The method of claim 8 in which the cells are fibroblasts.

10. A method for increasing the energy level in skin cells which comprises applying to the skin an effective amount of the composition of claim 2.

11. A method for increasing the energy level in skin cells which comprises applying to the skin an effective amount of the composition of claim 3.

12. A method for increasing the energy level in skin cells which comprises applying to the skin an effective amount of the composition of claim 4.

13. A method for increasing the energy level in skin cells which comprises applying to the skin an effective amount of the composition of claim 5.

14. A method for increasing the energy level in skin cells which comprises applying to the skin an effective amount of the composition of claim 6.

15. A method of treating or preventing symptoms of aging in the skin which comprises applying to the skin an effective amount of the composition of claim 1.

16. The method of claim 15 in which the symptom is skin atrophy.

17. The method of claim 15 in which the symptom is loss of skin thickness.

18. The method of claim 15 in which the symptoms are lines and wrinkles.

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/US 99/08497

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K7/48 A61K31/70 A61K31/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9625 Derwent Publications Ltd., London, GB; Class B02, AN 96-246892 XP002900578 & JP 08 099860 A (KOSE KK), 16 April 1996 (1996-04-16) abstract --- DATABASE WPI Section Ch, Week 9415 Derwent Publications Ltd., London, GB; Class B04, AN 94-121159 XP002900579 & JP 06 065041 A (KOSE KK), 8 March 1994 (1994-03-08) abstract --- -/--	1-18
X		1-8, 10-15

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
17 August 1999	27 09 1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 99/08497

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9423 Derwent Publications Ltd., London, GB; Class A96, AN 94-188881 XP002900580 & JP 06 128140 A (KOSE KK), 10 May 1994 (1994-05-10) abstract ---	1-6
X	DATABASE WPI Section Ch, Week 9734 Derwent Publications Ltd., London, GB; Class B04, AN 97-369368 XP002900581 & JP 09 157153 A (NOEVIR KK), 17 June 1997 (1997-06-17) abstract ---	1-5, 15-18
X	DATABASE WPI Section Ch, Week 9148 Derwent Publications Ltd., London, GB; Class B03, AN 91-350854 XP002900582 & JP 03 236320 A (KOBAYASHI KOSE KK), 22 October 1991 (1991-10-22) abstract -----	1-14

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/08497

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 7-18 are directed to a method of treatment of the human or animal body by therapy (PCT-Rule 39.1 (iv)) the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. ...nal Application No
PCT/US 99/08497

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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